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EFFECT OF TEMPERATURE AND SALINITY ON LARVAL DEVELOPMENT OF SIBLING SPECIES OF *ECHINASTER* (ECHINODERMATA: ASTEROIDEA) AND THEIR HYBRIDS

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ABSTRACT

Adult *Echinaster* Type 1 and Type 2 were collected along the west coast of Florida (25°C, 32‰S) and induced to spawn in the laboratory. Two-day old larvae of Type 1, Type 2, and their hybrids were subjected to temperature (T) and salinity (S) combinations (20, 25, 30°C; 25, 32, 39‰S). Response surface isopleths indicate that Type 1 and Type 2 larvae exhibit different developmental and growth rates in response to T/S combinations. Salinity was a dominant factor affecting development and growth. High and low salinities inhibited spine development. Developmental rates were directly related to temperature. Type 2 larvae exhibited a greater tolerance to temperature changes than did Type 1. Hybrids showed intermediate development and growth responses at the apparent optimal conditions and exhibited maternal characteristics.

INTRODUCTION

Sibling species (Campbell and Turner, 1979) of the asteroid *Echinaster* occur in the vicinity of Tampa Bay, Florida. One is blue-orange in color and the other orange-brown. Downey (1973) characterized both as morphs of *E. modestus* based on spination in dried specimens. However, Campbell and Turner (1979) noted morphological differences between the two and concluded that they were sibling species. Other characteristics of these species are reviewed by Scheibling and Lawrence (1982).

The species have been classified by Atwood (1973) according to egg type: Type 1 with buoyant eggs and larvae which are planktonic for approximately two days, Type 2 with demersal eggs and completely benthic larvae. Type 1 adults generally spawn in the spring 2–4 weeks after Type 2 (Scheibling and Lawrence, 1982). Although these *Echinaster* species are found in different habitats, they may potentially interbreed.

Phenotypic variation among individuals may reflect basic genetic differences or environmentally induced modifications and phenotypic plasticity (Marcus, 1980). Genetic differences between the two species of *Echinaster* may be reflected in their physiology. The genetic and environmental factors that control the growth and development of the species may be distinguished by rearing individuals under controlled laboratory conditions.

Hybridization of these species of *Echinaster* is possible by cross-fertilization in the laboratory (Scheibling, 1982), although there is no evidence that hybridization of the two morphs occurs in nature. Echinoderm hybridization between both genera and species has been documented in the laboratory (Tennent, 1910; Harvey, 1956; Hägstrom and Lönning, 1961; Hinegardner, 1967, 1975; Horstadius, 1973; Lucas

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and Jones, 1976; Strathmann, 1981) and in the field (Verrill, 1909; Hägstrom and Lönning, 1961; Strathmann, 1981). Genetic differences may be reflected by differential responses of the species and their hybrids to different temperatures and salinities. The purpose of this study was to examine the effects of temperature and salinity on the development and growth of sibling species of *Echinaster* and their hybrids.

MATERIALS AND METHODS

Individuals of *Echinaster* were collected in May and June, 1981 from the coastal waters of the eastern Gulf of Mexico: Type 1 from the Skyway Bridge at the mouth of Tampa Bay, Type 2 from the intercoastal waterway at Anna Maria Island. Individuals were maintained in the laboratory for 24 hours in aerated sea water at field temperature and salinity of 25°C and 32‰ S. Spawning was induced with 0.001 *M* 1-methyladenine in sea water either *in vivo* via intracoelomic injection, or *in vitro* using excised ovaries and testis. Eggs and sperm were pooled separately from 3–6 individuals. Eggs of each Type were both fertilized and cross-fertilized to produce 4 groups of zygotes: *Echinaster* Type 1 wild (Type 1♀ × ♂), *E.* Type 1 maternal hybrid (Type 1♀ × Type 2♂), *E.* Type 2 wild (Type 2♀ × ♂), and *E.* Type 2 maternal hybrids (Type 2♀ × Type 1♂). Eggs of each morph were fertilized less than two weeks prior to spawning in nature. The lecithotrophic embryos were maintained at field temperatures and salinities during early cleavage and gastrulation. After two days they developed into motile, modified brachiolarian larvae and were placed in experimental temperatures and salinities.

Larvae were subjected to temperature and salinity combinations in a 3 × 3 factorial design (20, 25, 30°C; 25, 32, 39‰ S). Ranges of temperatures and salinities experienced by *Echinaster* in the field are 10–33°C and 25–35‰ S. Preliminary experiments indicated that larval densities of 5–25 individuals per bowl had no effect on development and growth. Duplicate sets of 20 larvae were placed in 6 cm diameter glass bowls containing approximately 25 ml of filtered (0.45 μm Millipore filter) sea water at each temperature and salinity combination. Only one set of Type 2 hybrid larvae was used. Treatment salinities were obtained by the addition of distilled water or sea-salt to sea water. Temperature and salinity were controlled in constant environment chambers within 0.5°C and 1‰ S of selected values. Larvae were reared in the dark and water was changed daily. Treatment bowls were placed on rotators to insure adequate mixing.

The developmental stage of all larvae was monitored daily. Data represent pooled observations. The following stages were recorded: brachiolarian larvae, first appearance of the first and second pairs of tube feet, first appearance of the third pair of tube feet, first appearance of the fourth pair of tube feet, and the appearance of the mouth. All stages have been described and illustrated (Kempf, 1966; Atwood, 1973). In Figures 1 and 2 data represent the time of development reached by 75% of all individuals in a T/S combination. Radii of larval discs were measured at several stages. Growth was found to be linear by regression analysis. Average growth was measured by Δ radius/Δ time (days) × 100 ($n = 20$).

Response surface techniques (see Alderdice, 1972) were employed for statistical analysis of the effects of temperature and salinity combinations on larval development and growth. Isopleths were determined by a general linear model contained in the Statistical Analysis System (SAS). Approximate r^2 values between 0.81 and 0.98 indicate the data fit this model. Isopleths were extrapolated by the computer over the ranges of temperatures and salinities tested.

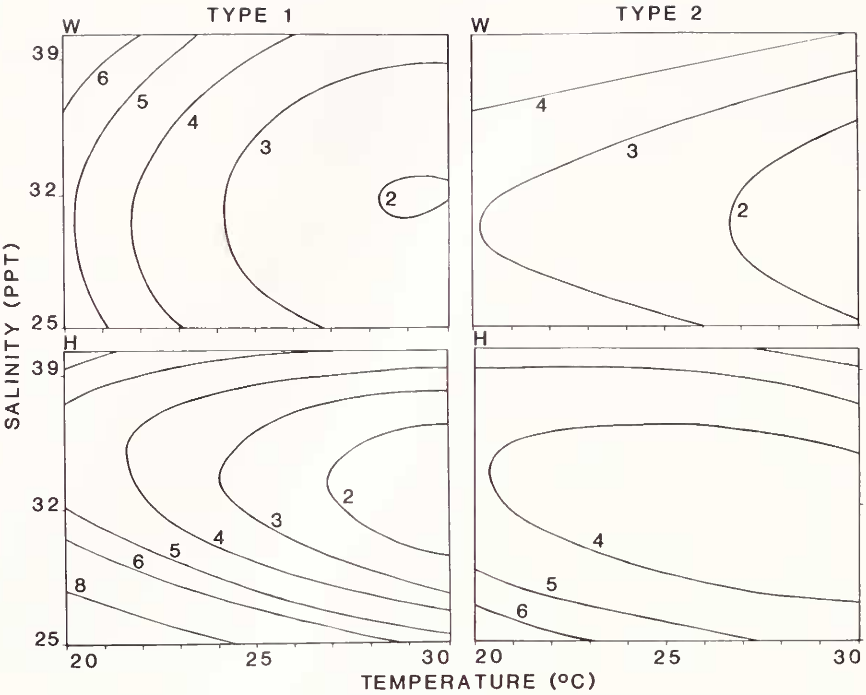

FIGURE 1. Response surface estimates of time (days) until the appearance of the first and second pairs of tube feet. Left column represents *Echinaster* Type 1 wild (W) and hybrid (H) individuals. Right column represents Type 2 wild and hybrid individuals.

RESULTS

Response surface models of temperature and salinity combinations on the time (days) until the appearance of the first and second pairs of larval tube feet for *Echinaster* Type 1, Type 2, and their hybrids are shown in Figure 1. The appearance of the first and second pairs of tube feet represent an early stage of development of the larvae. Type 2 wild developed faster than Type 1 wild. The apparent optimal conditions, *i.e.*, those resulting in the fastest rates of development, are not different at this stage. Small changes in salinity appear to have a pronounced effect on development, indicated by narrow isopleths. Hybrids show intermediate developmental rates at the apparent optimal conditions with the maternal influence being strongest. Hybrids developed more slowly than the maternal parent at the extreme temperature and salinity combinations.

Response surface models of temperature and salinity combinations on time (days) until the appearance of the mouth are shown in Figure 2. This represents a late stage in the development of the juvenile just prior to feeding. The mouth of *Echinaster* Type 1 wild generally appears earlier than Type 2 wild. Distinct differences were seen in the apparent optimal conditions. Hybrids generally exhibit a dominant maternal influence. At this stage Type 1 hybrids exhibited faster rates of development than the maternal parent, and Type 2 hybrid rates were slower than the maternal parent.

Response surface models of temperature and salinity combinations on growth rates are shown in Figure 3. Growth rates were faster in Type 2 individuals and

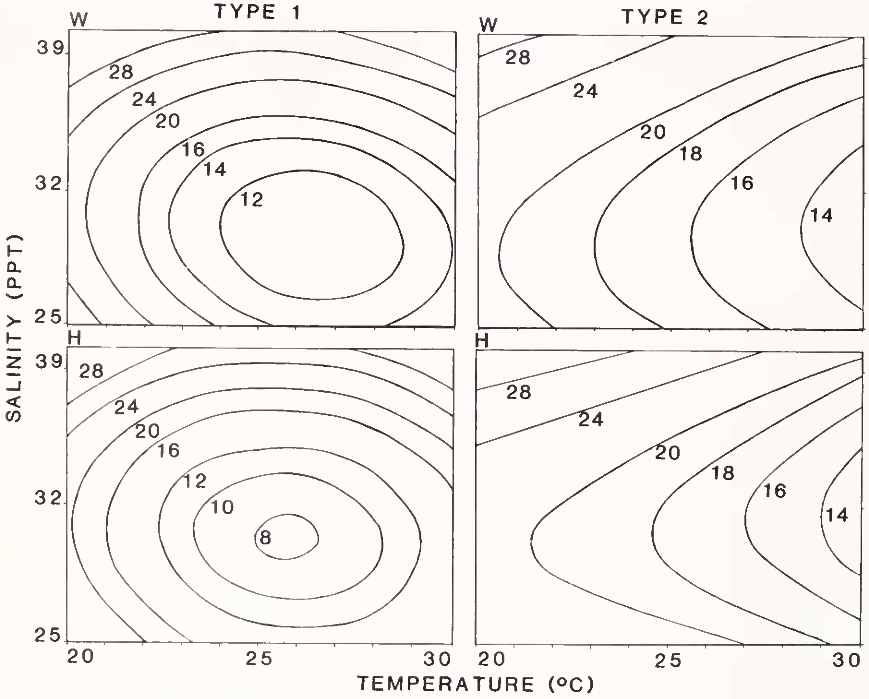

FIGURE 2. Response surface estimates of time (days) until the appearance of the mouth. Left column represents *Echinaster* Type 1 wild (W) and hybrid (H) individuals. Right column represents Type 2 wild and hybrid individuals.

correspond with faster development (Figures 1 and 2). Apparent optimal conditions differed between Type 1 and Type 2 wild. Salinity changes had a pronounced effect on growth. Isopleths along the temperature axis indicated the eurythermality of both types, with Type 2 being more eurythermal than Type 1. Maternal hybrids exhibited intermediate growth rates at the optimal temperature and salinity combinations.

Initial exposure of the larvae to T/S combinations induced negligible mortality in all combinations (<1%). Greatest mortality (20%) occurred after larvae developed the mouth, and was presumed to result from lack of food. There was no higher mortality among hybrids.

Abnormal spine development was observed in several larvae exposed to high and low salinities. In these organisms there was little or no development of mouth-frame armature spines or of the terminal spines of the rays, as observed by light microscopy. Several larvae exposed to high salinity exhibited abnormal ray number development.

DISCUSSION

Temperature and salinity are of primary importance in determining larval development and survival in marine habitats. The importance of the combined effects of temperature and salinity has been emphasized by Kinne (1970). These factors are particularly important to larvae that may encounter a variable environment, as the range of environmental factors tolerated by larvae and juveniles is usually narrower than that tolerated by the adult (Vernberg and Vernberg, 1975).

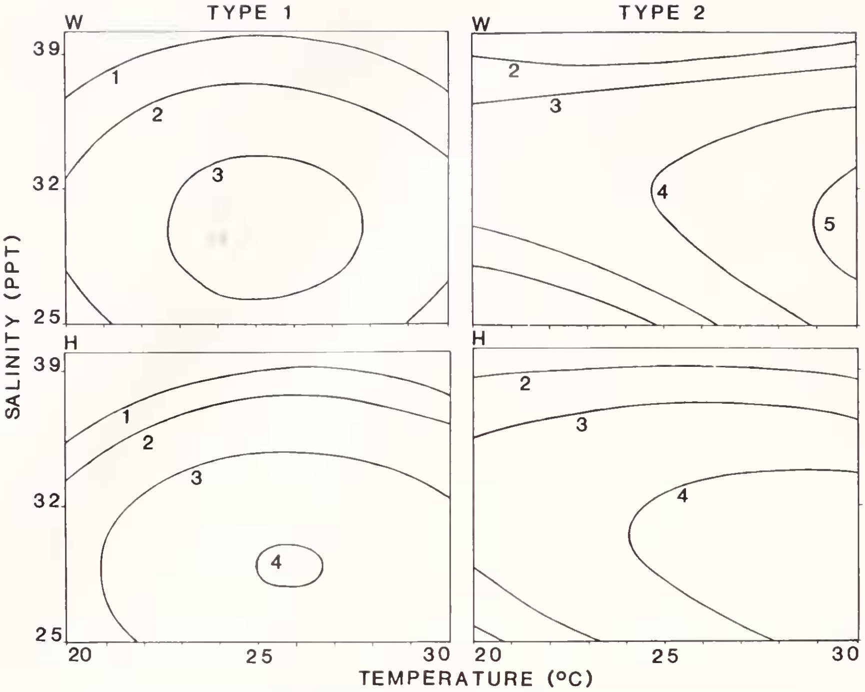

FIGURE 3. Response surface estimates of growth rates. Large values indicate faster rates of growth. Left column represents *Echinaster* Type 1 wild (W) and hybrid (H) individuals. Right column represents Type 2 wild and hybrid individuals.

Within the thermal tolerance limits of the species, warmer temperatures normally accelerate the growth processes. Development rates of larval *Echinaster* Types 1 and 2 were directly related to temperature. This was expected, as metabolic rate increases with temperature in larvae of marine invertebrates in general (Kinne, 1970).

Salinity greatly influenced development in *Echinaster* Types 1 and 2. Optimal development and growth of both Types 1 and 2 occurred at 28–32‰ S. In both types poor spine development occurred in many individuals exposed to low and high salinities. In many individuals there was little or no development of the mouth-frame armature spines or of the terminal spines of the rays. This indicates that salinity may affect the deposition of carbonate material during spine formation. Metabolism associated with carbonate deposition may be sensitive to changing salinities and not due to the concentrations of Ca^{++} and Mg^{++} in the water, as poor spine formation occurred at both low and high salinities. In addition, several larvae exposed to high salinities exhibited abnormal ray number development, *i.e.*, 3- or 4-rayed instead of the usual 5 (Watts *et al.*, 1983).

The first and second tube feet pairs developed earlier in Type 2 wild individuals than Type 1 individuals. This may be adaptive to Type 2 larvae which generally inhabit shallow-water seagrass beds subjected to wave action. Apparent optimal temperatures and salinities between Type 1 and 2 do not differ at this early stage of development. However, low temperature has a more pronounced effect on Type 1 than Type 2 as indicated by slower developmental rates in Type 1 larvae.

The mouth generally developed earlier in Type 1 wild individuals than in Type 2. The mouth in Type 1 appears after the third pair of tube feet while the mouth in Type 2 does not appear until after the fourth pair of tube feet. The differences in developmental rates are similar to those found by Atwood (1973) and Kempf (1966). Distinct differences were seen in the apparent optimal temperatures and salinities at this stage. At high temperatures, Type 2 individuals developed faster than did Type 1, and these differences are also shown in faster growth rates of Type 2 larvae. However, once the mouth is present and the juveniles begin to feed, Type 1 grows more rapidly than Type 2 (Scheibling, 1982).

Type 2 appears to be more eurythermal than Type 1 and this may be reflective of its shallow water existence. Type 1 generally inhabits deeper water than Type 2 (Atwood, 1973). The greater tolerance of Type 2 for a wider range of temperatures in shallow water may be related to past selective influences by the thermal environment on previous generations. Larval development and growth rates may also be influenced by environmental conditions experienced by the adults, particularly during gonadal development (Davies, 1958). Thermal tolerance of aquatic poikilotherms is in part dependent on their thermal history (Kinne, 1970).

Hybrids of *Echinaster* Type 1 and Type 2 generally exhibited intermediate developmental and growth rates at the apparent optimal temperatures and salinities. Type 1 hybrids at time of the appearance of the mouth exhibited faster developmental and growth rates than the maternal parent, while Type 2 hybrids exhibited slower developmental and growth rates than their maternal parent. This indicates that developmental and growth rates are directly related, but separate phenomena.

These factors suggest that the differences in temperature and salinity responses between the two morphs are genetically controlled, and are not environmentally influenced. The maternal influence is apparently stronger in the hybrids than the paternal influence. Andronikov (1967) found that the zygote obtained by fertilization of *Strongylocentrotus nudus* ova via *S. intermedius* spermatozoa showed the same level of heat tolerance as a normal *S. nudus* zygote, indicating the dominant influence of the eggs' thermal characteristics. Hinegardner (1975) crossed the sand dollars *Encope californicus* and *Dendraster excentricus* and reported that during accidental exposure of larvae to high temperatures, physiological responses to stress were inherent in the hybrids. Furthermore, Hinegardner found that the hybrids exhibited paternal characteristics, which is considered to be a general tendency of echinoderm hybrids. Lucas and Jones (1976) found that hybrids of the asteroids *Acanthaster planci* and *A. brevispinus* were morphologically intermediate. Marcus (1980) also found intermediate differences indicating joint influences of maternal and paternal genomes in the echinoid *Arbacia punctulata* from two geographic localities. Scheibling (pers. obs.) found that Type 2 hybrids reared in the laboratory morphologically resemble the maternal parent. Cytoplasmic constituents in the eggs may influence the developing larvae in *Echinaster*. The genetic influence of the maternal and paternal genomes is apparently variable in echinoderms.

The differences in the development and growth of the sibling species of *Echinaster* appear to be genetically controlled, and not the result of environmentally induced modifications of their physiology. Although most echinoderm hybrids do not live beyond the larval stage (Hinegardner, 1975), Scheibling (1982) reared Type 2 hybrids for one year in the laboratory and found their growth rates to either equal or exceed that of Type 2 wild individuals. The high degree of genetic compatability between the two species, as demonstrated by the F_1 hybrids, indicate that the two species are closely related and suggests recent speciation.

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LITERATURE CITED

ALDERDICE, D. F. 1972. Factor combinations. Responses of marine poikilotherms to environmental factors acting in concert. Pages 1659–1722 in *Marine Ecology*, O. Kinne, Ed. Wiley, London.

ANDRONIKOV, V. B. 1967. Heat-resistance of gametes of poikilothermic animals. Pages 398–402 in *The Cell and Environmental Temperature*, A. S. Troshin, Ed. Pergamon Press, New York.

ATWOOD, D. G. 1973. Larval development in the asteroid *Echinaster echinophorus*. *Biol. Bull.* **144:** 1–11.

CAMPBELL, D. B., AND R. L. TURNER. 1979. Comparative skeletal and ossicle morphology of sibling species of *Echinaster* (Echinodermata: Asteroidea) from the west coast of Florida. *Am. Zool.* **19:** 1009.

DAVIES, H. C. 1958. Survival and growth of clam and oyster larvae at different salinities. *Biol. Bull.* **114:** 296–307.

DOWNEY, M. E. 1973. Starfish from the Caribbean and Gulf of Mexico. *Smithson. Contrib. Zool.* **126:** 1–158.

HAGSTRÖM, B. E., AND S. LÖNNING. 1961. Morphological and experimental studies on the genus *Echinus*. *Sarsia* **4:** 21–31.

HARVEY, E. B. 1956. The American *Arbacia* and other sea urchins. Princeton Univ. Press, Princeton, New Jersey.

HINEGARDNER, R. T. 1967. *Echinoderms*. Pages 139–155 in *Methods in Developmental Biology*, F. H. Wilt and N. K. Wessels, Eds. T. Y. Cromwell Co., New York.

HINEGARDNER, R. T. 1975. Morphology and genetics of sea urchin development. *Am. Zool.* **15:** 679–690.

HORSTADIUS, S. 1973. *Experimental embryology of echinoderms*. Clarendon Press, Oxford.

KEMPF, M. 1966. On the development of *Echinaster echinophorus* (Lmk.). *Academia Brasileira de Ciencias, Annaes* **38:** 505–507.

KINNE, O. 1970. Environmental factors: temperature. Pages 321–616 in *Marine Ecology*, O. Kinne, Ed. Vol. 1. Wiley-Interscience, London.

LUCAS, J. S., AND M. M. JONES. 1976. Hybrid crown-of-thorns starfish (*Acanthaster planci* × *A. brevispinus*) reared to maturity in the laboratory. *Nature* **263:** 409–412.

MARCUS, N. H. 1980. Genetics of morphological variation in geographically distant populations of the sea urchin *Arbacia punctulata* (Lamarck). *J. Exp. Mar. Biol. Ecol.* **43:** 121–130.

SCHEIBLING, R. E. 1982. Differences in body size and growth rate between morphs of *Echinaster* (Echinodermata: Asteroidea) from the eastern Gulf of Mexico. In press in *International Echinoderms Conference-Tampa Bay*, J. M. Lawrence, Ed. A. A. Balkema, Roterdam.

SCHEIBLING, R. E., AND J. M. LAWRENCE, 1982. Differences in reproductive strategy between morphs of *Echinaster* (Echinodermata: Asteroidea) from the eastern Gulf of Mexico. *Mar. Biol.* In press.

STRATHMANN, R. R. 1981. On barriers to hybridization between *Strongylocentrotus droebachiensis* (O. F. Müller) and *S. pallidus* (G. O. Sars). *J. Exp. Mar. Biol. Ecol.* **55:** 39–47.

TENNENT, D. H. 1910. Echinoderm hybridization. *Carnegie Inst. Wash.* **132:** 117–152.

VERNBERG, F. J., AND W. B. VERNBERG. 1975. Adaptations to extreme environments. Pages 165–180 in *Physiological Ecology of Estuarine Organisms*, F. J. Vernberg, Ed. Univ. South Carolina Press, Columbia, South Carolina.

VERRILL, A. E. 1909. Remarkable development of starfishes on the Northwest American coast; hybridization; multiplicity of rays; teratology; problems in evolution; geographical distribution. *Am. Nat.* **43:** 542–555.

WATTS, S. A., R. E. SCHEIBLING, A. G. MARSH, AND J. B. MCCLINTOCK. 1983. Induction of aberrant ray numbers in *Echinaster* sp. (Echinodermata: Asteroidea) by high salinity. *Florida Scientist* **46:** in press.